pET24a *E. coli* expression system. The C-terminus peptide produced a very high titer antiserum ($\alpha$-C1) that contains significant activity at 1:50,000 dilution. The $\alpha$-C1 antiserum was particularly rigorous and retained activity in detergent conditions up to 1% SDS. This antibody is satisfactory for Western blot detection of the recombinant human leptin and the recombinant porcine leptin (FIG. 10). This antibody is used for Western blot detection of the recombinant human leptin (lanes 1-4, 14.8 kDa) obtained from Eli Lilly & Co., and the recombinant porcine leptin (lanes5-8, 16 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa. The data presented are a concentration curve (.05 - 1 ìg) of each protein.

Q2

## REMARKS

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date February 13, 2003

By Alan E. Wagner
Alan E. Wagner
Registration No. 45188

**WHYTE HIRSCHBOECK DUDEK S.C.**
111 East Wisconsin Avenue, Suite 2100
Milwaukee, Wisconsin 53202
Customer No. 022202

**MARKED UP VERSION ATTACHED TO AMENDMENT IN SERIAL NO. 09/932,888**

**Marked up version of the paragraph on page 29, lines 1-21 thru page 30, lines 1-3,is below:**

A porcine *ob* (*obese* gene) cDNA probe was amplified from adipose tissue mRNA using the reverse transcriptase-polymerase chain reaction (RT-PCR). First strand cDNA synthesis reactions were carried out using 1-2 μg of porcine adipose tissue total RNA, 150 pmol of random hexamer oligonucleotides, 500 nM dNTP, 200 U of Superscript II reverse transcriptase (LifeTechnologies, Inc., Bethesda, MD, USA) in 20 μl of the supplied buffer. The reactions were incubated for 1 h at 37 °C and terminated by heating to 70°C for 10 min. The *ob* cDNA product was amplified by PCR using the following degenerate primers with restriction site linkers for BamHI and XbaI respectively; sense strand 5'-GTGCCYATCCARAAAGTCC-3' **(SEQ ID NO: 9)** and antisense strand 5'-GCAYYCAGGGCTRASRTC-3' **(SEQ ID NO: 10)**. Adipose tissue cDNA was added as template to 50 μl PCR reactions made in the manufacturer's buffer with 100 pmol of each primer and 2.5 U of Taq DNA polymerase (LifeTechnologies, Inc.). A three stage amplification was carried out under the following conditions; Stage 1- 95°C, 3 min; 52°C, 1 min; 72°C, 1 min; 1 cycle; Stage 2- 94°C, 45s; 52°C, 45s; 72°C, 1 min; 4 cycles; Stage 3- 94°C, 45 s; 55°C, 30 s; 72°C 1 min; 28 cycles. The PCR products were digested with the restriction enzymes BamHI and Xba I and purified by electrophoresis in 1% NuSieve low melting point agarose (FMC Bioproducts, Rockland, ME, USA). The *ob* cDNA was ligated into Bluescript II SK+ (Stratagene Inc. LaJolla, CA, USA) and transformed into MCR DH5α (LifeTechnologies, Inc.) and plated on LB plates containing 50 μg/ml ampicillin for plasmid selection. Twelve *E. coli* colonies were isolated that contained the porcine *ob* cDNA and plasmid DNA was isolated for sequencing. Dideoxy sequencing reactions were carried out using [$^{35}$S] dATP labeling with Sequenase V2.0. The sequence samples were loaded on 5% Long Ranger (FMC Bioproducts) for denaturing gel electrophoresis according to the manufacturer's recommendations.

**Marked up version of paragraph on page 32, lines 7-19, is below:**

Rabbit polyclonal antibodies were initially made to synthetic peptides derived from the N-terminus of the secreted portion of the protein (WRVQDDTKTLIKTIVTRISD) **(SEQ ID NO: 11)** as a map peptide and the C-terminus (C1 peptide, LQGALQDMLRQLDLSPGC) **(SEQ ID NO: 12)** for conjugation to keyhole limpet hemocyanin. Both peptides produced antibodies in rabbits that cross-react with the recombinant pig leptin produced using the pET24a *E. coli* expression system. The C-terminus peptide produced a very high titer antiserum ($\alpha$-C1) that contains significant activity at 1:50,000 dilution. The $\alpha$-C1 antiserum was particularly rigorous and retained activity in detergent conditions up to 1% SDS. This antibody is satisfactory for Western blot detection of the recombinant human leptin and the recombinant porcine leptin (FIG. 10). This antibody is used for Western blot detection of the recombinant human leptin (lanes 1-4, 14.8 kDa) obtained from Eli Lilly & Co., and the recombinant porcine leptin (lanes5-8, 16 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa). Lanes 7 and 8 reveal a dimer at approximately 33 kDa. The data presented are a concentration curve (.05 - 1 ig) of each protein.